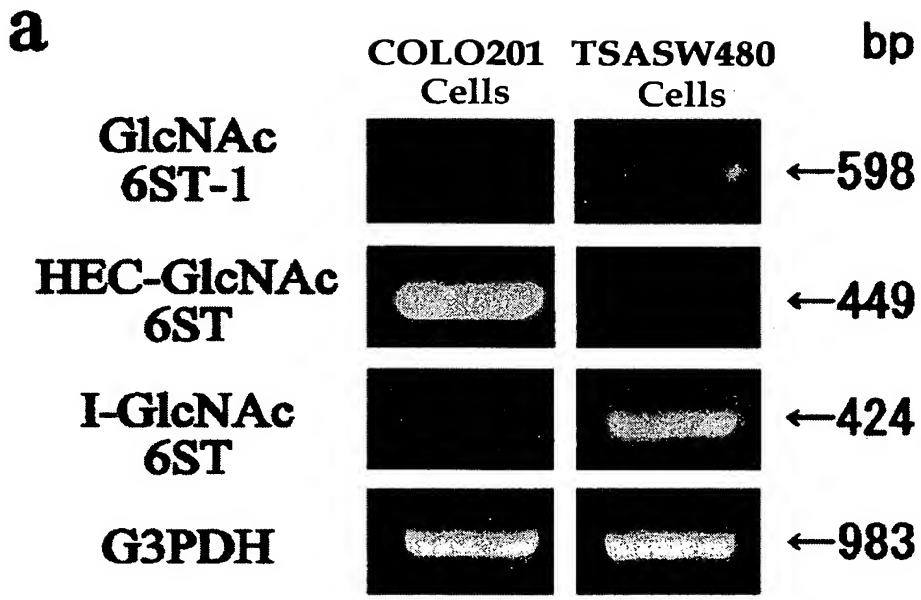
Figure 1


a
COLO201 Cells
TSASW480 Cells
bp
GlcNAc 6ST-1
←598
HEC-GlcNAc 6ST
←449
I-GlcNAc 6ST
←424
G3PDH
←983

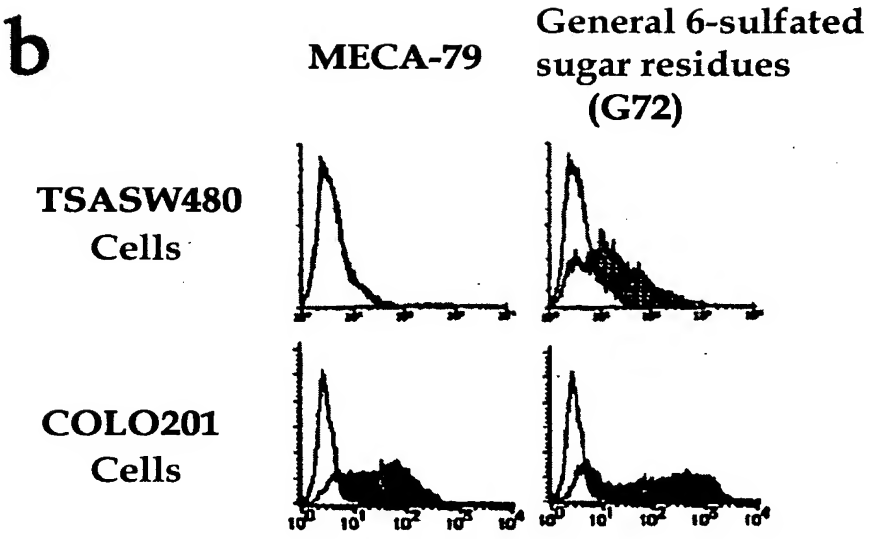

b
MECA-79
General 6-sulfated sugar residues (G72)
TSASW480 Cells
COLO201 Cells

Figure 2

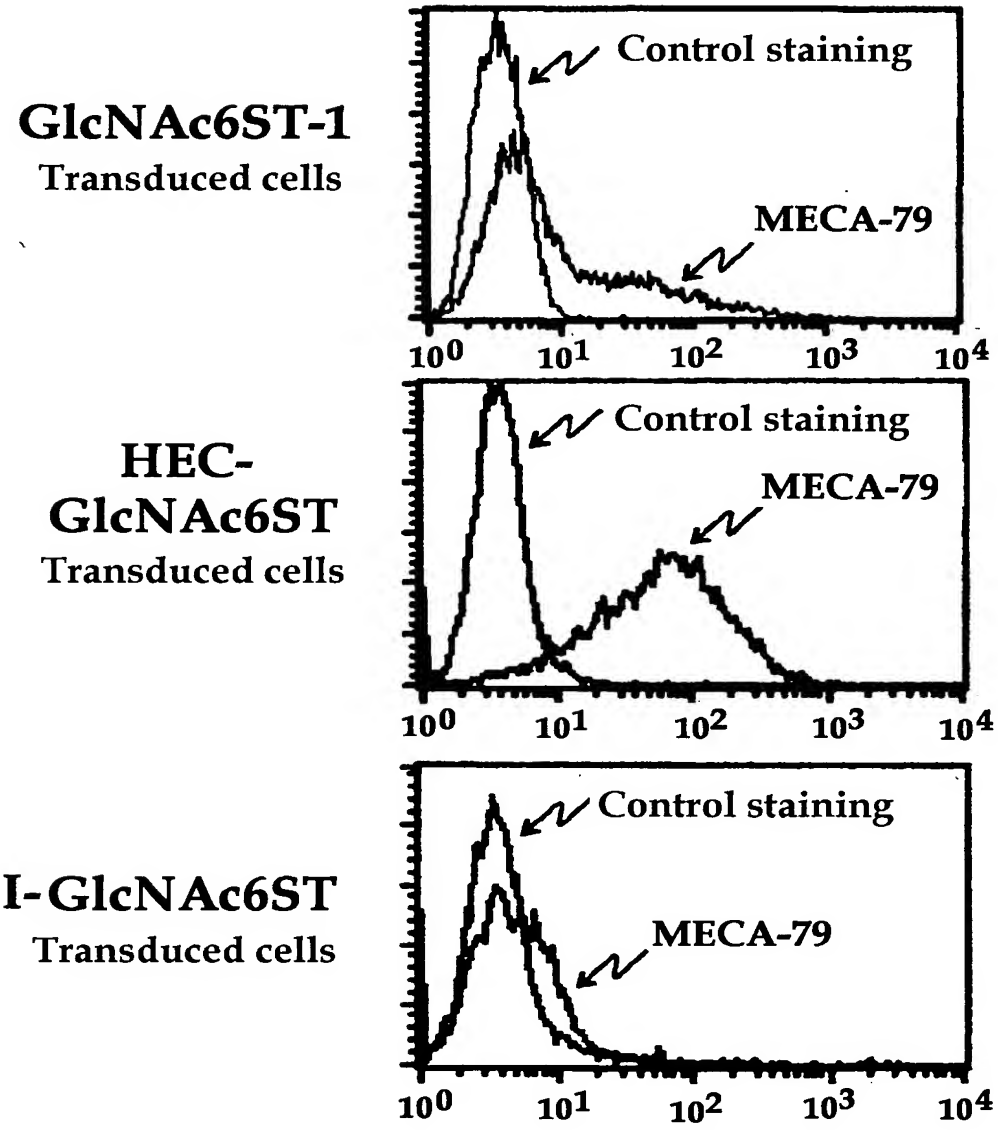

GlcNAc6ST-1
Transduced cells
Control staining
MECA-79
10^0 10^1 10^2 10^3 10^4
HEC-GlcNAc6ST
Transduced cells
Control staining
MECA-79
10^0 10^1 10^2 10^3 10^4
I-GlcNAc6ST
Transduced cells
Control staining
MECA-79
10^0 10^1 10^2 10^3 10^4

Figure 3

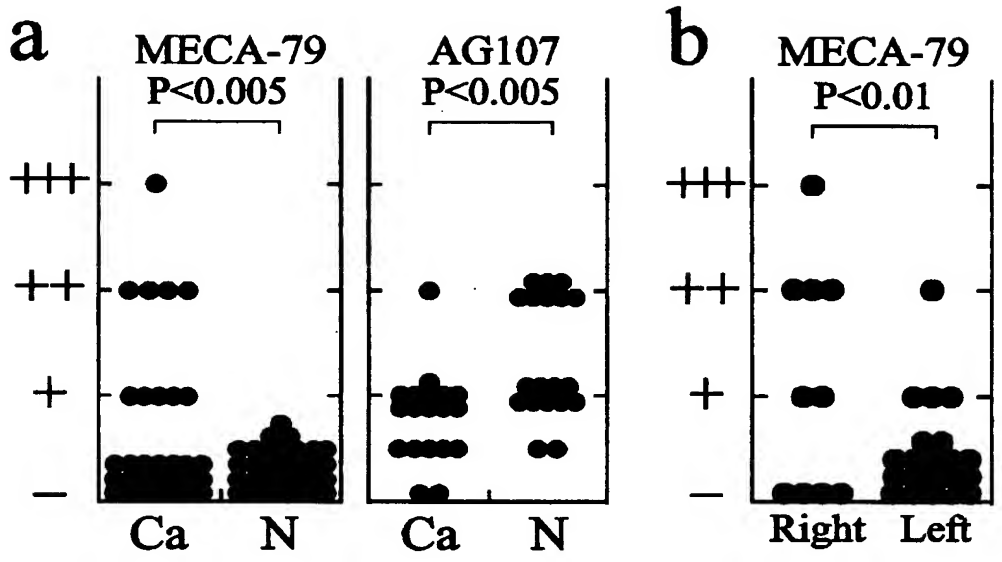


Figure 4

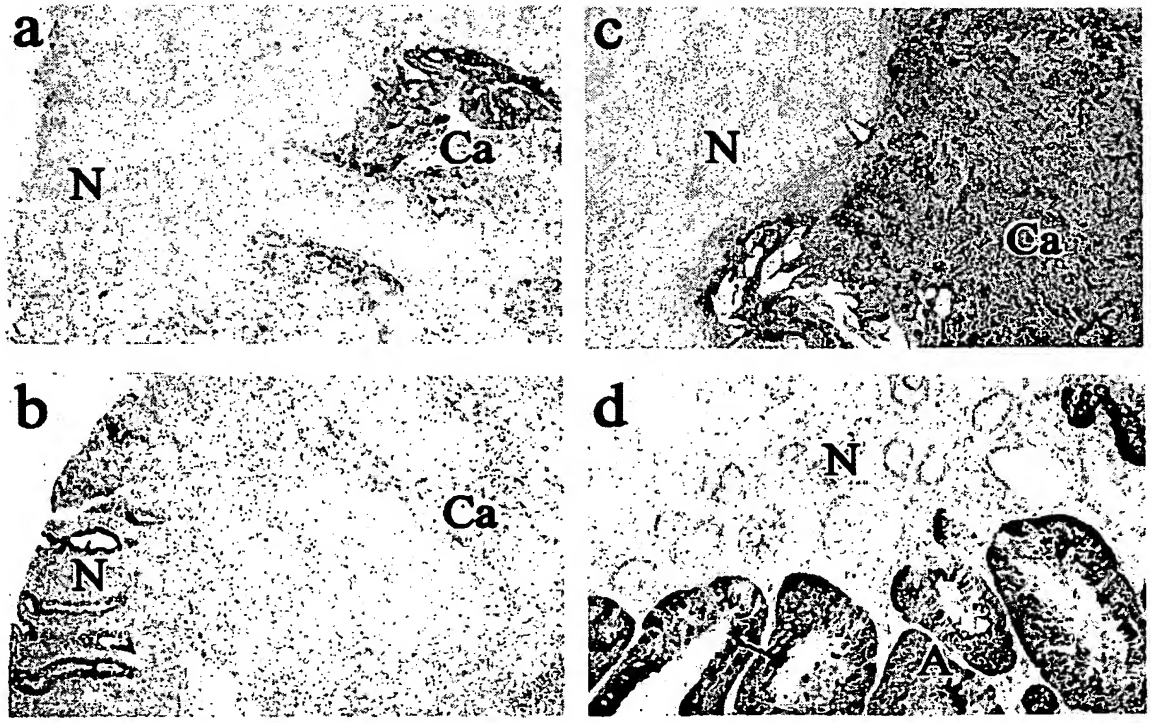


Figure 5

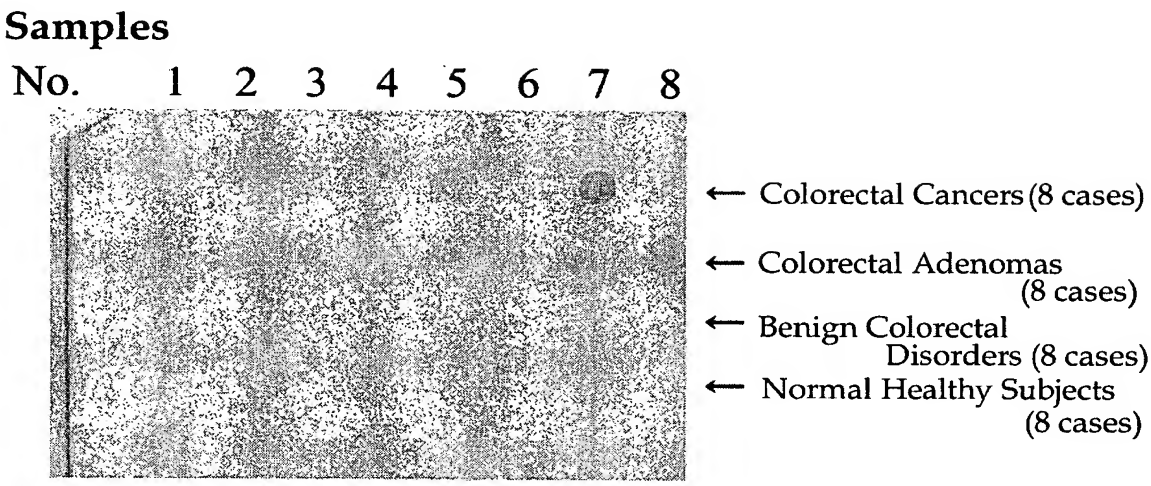

Samples
No. 1 2 3 4 5 6 7 8
← Colorectal Cancers (8 cases)
← Colorectal Adenomas (8 cases)
← Benign Colorectal Disorders (8 cases)
← Normal Healthy Subjects (8 cases)

Figure 6

BD PharMingen Technical Data Sheet

PURIFIED RAT ANTI-MOUSE PNAd CARBOHYDRATE EPITOPE (CD62L Ligand) MONOCLONAL ANTIBODY

PRODUCT INFORMATION

Catalog Number:	**553863** (Was: 09961D), **0.5 mg**
Description:	Purified anti-mouse PNAd Carbohydrate Epitope (CD62L Ligand)
Clone:	MECA-79
Immunogen:	Collagenase-dispersed BALB/c lymph node stroma[1]
Isotype:	Rat (Wistar) IgM, κ
Contents:	Purified immunoglobulin in 10 mM phosphate buffer, pH 7.2 with 500 mM NaCl and 0.09% (w/v) sodium azide.

SPECIFICITY

The MECA-79 antibody reacts with sulfate-dependent carbohydrate epitopes of peripheral lymph node addressin (PNAd).[2] The MECA-79–reactive antigen is closely associated with the carbohydrate ligands for L-selectin (*e.g.*, CD34, GlyCAM-1, MAdCAM-1), which are expressed on high endothelial venules (HEV) in lymphoid tissues and at sites of chronic inflammation.[1,2,3,4,5,6] Cross-reactivity with human,[3,4] ovine,[7] bovine,[7] primate,[7] and porcine[8] tissues has been observed. MECA-79 antibody inhibits L-selectin– dependent lymphocyte and platelet homing to lymph nodes *in vivo*[1,9] and *in vitro* adhesion to lymphoid tissue HEV[1,4] and immobilized PNAd.[3,9,10]

PREPARATION AND STORAGE

The antibody was purified from tissue culture supernatant by affinity chromatography. The antibody solution should be stored undiluted at 4°C.

USAGE

This antibody has been tested by immunohistochemical staining (IHC) of citrate-pretreated formalin-fixed paraffin-embedded sections (5 - 20 µg/ml) to assure specificity and reactivity. Other reported applications include IHC of acetone-fixed frozen sections,[1,4,5] immunoprecipitation[2,3] western blot analysis,[10] and *in vitro* and *in vivo* adhesion blocking.[1,3,4,9,10] **Since applications vary, each investigator must determine dilutions appropriate for individual use.**

Caution: Sodium azide is a reversible inhibitor of oxidative metabolism; therefore, antibody preparations containing this preservative agent must not be used in cell cultures nor injected into animals. Sodium azide may be removed by washing stained cells or plate-bound antibody or dialyzing soluble antibody in sodium azide-free buffer. Since endotoxin may also affect the results of functional studies, we recommend the NA/LE™ (No Azide/Low Endotoxin) antibody format for *in vitro* and *in vivo* use.

REFERENCES

1. Streeter, P.R., B.T.N. Rouse, and E.C. Butcher. 1988. Immunohistologic and functional characterization of a vascular addressin involved in lymphocyte homing into peripheral lymph nodes. *J. Cell Biol.* 107: 1853 - 1862.
2. Hemmerich, S, E.C. Butcher, and S.D. Rosen. 1994. Sulfation-dependent recognition of high endothelial venules (HEV)-ligands by L-selectin and MECA 79, an adhesion-blocking monoclonal antibody. *J. Exp. Med.* 180: 2219 - 2226.
3. Berg, E.L., M.K. Robinson, R.A. Warnock, and E.C. Butcher. 1991. The human peripheral lymph node vascular addressin is a ligand for LECAM-1, the peripheral lymph node homing receptor. *J. Cell Biol.* 114: 343 - 349.
4. Michie, S.A., P.R. Streeter, P.A. Bolt, E.C. Butcher, and L.J. Picker. 1993. The human peripheral lymph node vascular addressin. An inducible endothelial antigen involved in lymphocyte homing. *Am. J. Pathol.* 143: 1688 - 1698.
5. Faveeuw, C., M.-C. Gagnerault, and F. Lepault. 1994. Expression of homing and adhesion molecules in infiltrated Islets of Langerhans and salivary glands of nonobese diabetic mice. *J. Immunol.* 152: 5969 - 5978.

Please see Page 2.

Figure 7

REFERENCES (Continued)

6. Maly, P., A.D. Thall, B. Petryniak, C.E. Rogers, P.L. Smith, R.M. Marks, R.J. Kelly, K.M. Gersten, G. Cheng, T.L. Saunders, S.A. Camper, R.T. Camphausen, F.X. Sullivan, Y. Isogai, O. Hindsgaul, U.H. von Andrian, and J.B. Lowe. 1996. The α(1,3)fucosyltransferase Fuc-TVII controls leukocyte trafficking through an essential role in L-, E-, and P-selectin ligand biosynthesis. *Cell* 86: 643 - 653.
7. Butcher, E.C. Personal communication.
8. Binns, R.M., A. Whyte, S.T. Licence, A.A. Harrison, Y.T.M. Tsang, D.O. Haskard, and M.K. Robinson. 1996. The role of E-selectin in lymphocyte and polymorphonuclear cell recruitment into cutaneous delayed hypersensitivity reactions in sensitized pigs. *J. Immunol.* 157: 4094 – 4099.
9. Diacovo, T.G., K.D. Puri, R.A. Warnock, T.A. Springer, and U.H. von Andrian. 1996. Platelet-mediated lymphocyte delivery to high endothelial venules. *Science* 273: 252 - 255.
10. Puri, K.D., E.B. Finger, G. Gaudernack, and T.A. Springer. 1995. Sialomucin CD34 is the major L-selectin ligand in human tonsil high endothelial venules. *J. Cell Biol.* 131: 261 - 270.